Food Chemistry **24** (1987) 219–226

Properties of Winged Bean Lipoxygenase *(Psophocarpus tetragonolobus)*

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(Received 9 July 1986; revised version received 7 August 1986; accepted 29 September 1986)

ABSTRACT

Several properties of winged bean lipoxygenase including effects of pH, linoleic acid concentration and heat-treatment have been investigated. Optimal activity was found at pH 9 and a linoleic acid concentration of approximately $1{\cdot}7 \times 10^{-3}$ M. *The crude extract was relatively stable at 50°C but lost half its activity within 5 min at 60°C and 1 min at 65°C. These results have been compared with the literature, and differences have been discussed. Effects of heat-treatment on whole winged bean seeds have also been investigated. Lipoxygenase activity was completely eliminated by boiling the seeds in water for 10 min but inactivation was considerably slower during dry heating. Peroxidase was shown to be significantly more stable to heat-treatment than lipoxygenase. Trypsin inhibitor activity and nitrogen extractability were also monitored.*

INTRODUCTION

Seeds of the winged bean (*Psophocarpus tetragonolobus*) have a high content of both protein (28–47%) and oil (15–28%) (Khor *et al.*, 1982). Consequently the bean is a promising crop for the humid tropics where soybeans do not grow well. Winged bean seeds are susceptible to oxidative rancidity due to the high linoleic acid content (Bodger *et al.*, 1982) and the presence of lipoxygenase (Truong *et al.*, 1982*a*). Two major lipoxygenase isoenzymes

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have been isolated (Truong *et al.*, 1982*a*), and their properties have been studied (Truong *et al.*, 1982*b*). The optimal pH for lipoxygenase activity was found to be pH 6·0 and 5·8 for these two isoenzymes, but these values contrast with the optimal pH of 9 reported by King and Puwastien (1984). These authors reported significant differences in the thermal stability of lipoxygenase from different varieties of winged bean. The present paper reports a further investigation of the properties and thermal stability of winged bean lipoxygenase. Since peroxidase is often monitored as a measure of residual enzymic activity in heat-treated foods, the thermal stability of lipoxygenase was compared with that of peroxidase. Trypsin inhibitor activity and nitrogen extractability were also monitored as measures of nutritional quality and functionality respectively.

MATERIALS AND METHODS

The winged bean seeds comprised a freshly harvested commercial seed mixture imported from Sri Lanka by Kins Plants Limited. Linoleic acid (99%) and Tween 20 were purchased from Sigma Chemical Company Limited. The winged bean seeds were ground into a flour, defatted with hexane and extracted with sodium phosphate buffer (0·1 M, pH 6·5) at 0°C. The crude lipoxygenase extract was filtered, centrifuged and treated with ammonium sulphate as described in an earlier publication (Mtebe & Gordon, 1987). Lipoxygenase activity was assayed at 234 nm by a spectrophotometric procedure based on that of Ben Aziz *et al.* (1970). Unit lipoxygenase activity corresponds to a change in absorbance of 0·001 min^{-1}. Moisture content was determined by the IUPAC procedure (IUPAC, 1979).

Peroxidase activity was determined by spectrophotometric assay at 420 nm, using *o*-phenylenediamine and hydrogen peroxide as described by Mihalyi & Vamos-vigyazo (1975). A change in absorbance of $1 \times 10^{-3} min^{-1}$ corresponds to unit activity. Trypsin inhibitor activity was determined by spectrophotometry at 410 nm, as described by Smith *et al.* (1980). N-Benzoyl-DL-arginine-*p*-nitroanilide was used as the substrate. Nitrogen extractability was determined by the method of Rivas *et al.* (1981). Measurements of lipoxygenase activity, peroxidase activity, trypsin inhibitor activity and nitrogen extractability were performed in duplicate and the mean value is quoted.

Thermal treatment of winged bean seeds involved immersing a sample (200 g) in water at 100°C or heating in an oven at 100°C. Treatment times in the oven represent the time after the oven regained the set temperature, which required 20 min approximately. Samples were removed from the heating medium, cooled at room temperature and stored at −20°C.

Heat-treatment of lipoxygenase extracts was performed by immersing test

tubes containing 5 ml aliquots (pH 7·0) in a water bath at the set temperature. Samples (1 ml) were removed periodically from the enzyme assay.

RESULTS AND DISCUSSION

Effect of pH and substrate concentration on lipoxygenase activity

Winged bean lipoxygenase activity was observed to be maximal at pH 9 at linoleic acid concentrations up to 2·64 mM (Fig. 1), but at higher concentrations the maximum activity shifted to pH 8 due to a strong reduction in activity at pH 9. The maximum activity at pH 9 occurred at a linoleic acid concentration of approximately $1{\cdot}7 \times 10^{-3}$ M. The spectrophotometric assay for lipoxygenase activity of crude extracts can be affected by enzymes which catalyse various hydroperoxide degradation reactions (Galliard & Chan, 1980). However, the lipoxygenase activity was still higher in an alkaline medium after subjecting the sample to gel chromatography. The activity of the most active fraction was 1500 units g^{-1} of defatted flour at pH 9 compared with 375 units g^{-1} at pH 6·2 after gel chromatography. While volatiles formed by decomposition of hydroperoxides were detected after incubation at both pH 6·5 and 9, the concentration of volatiles was

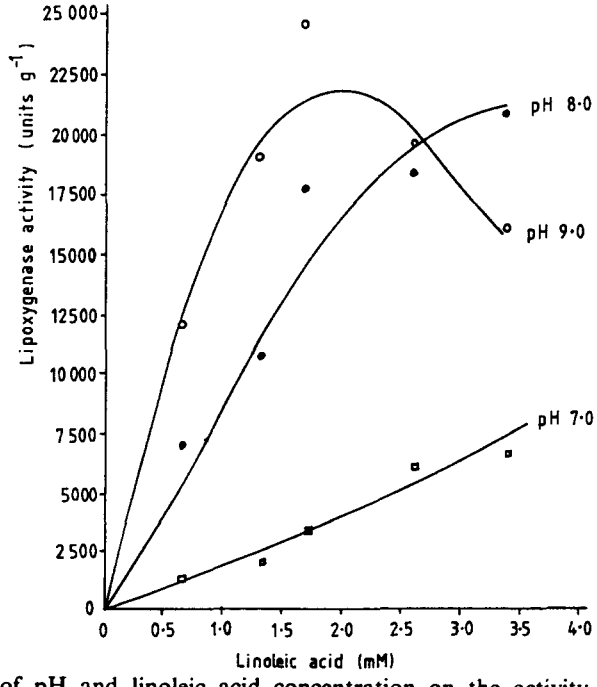

Fig. 1. Effect of pH and linoleic acid concentration on the activity of winged bean lipoxygenase.

about six times greater after incubation at pH 9 (Mtebe & Gordon, 1987). Hence, the observed large increase in lipoxygenase activity at an alkaline pH is not due to enhanced decomposition of hydroperoxides to form volatiles at neutral pH. It is therefore concluded that the lipoxygenase activity was maximal at pH 9. The activity at pH 9 was 7·5 times that at pH 7 with a substrate concentration of $1{\cdot}7 \times 10^{-3}$ M.

Optimum activity at pH 9 is characteristic of Type-1 lipoxygenase which is present in soybeans, but it contrasts with Type-2 lipoxygenase, found in a wide variety of plants including peas and corn, which has optimum activity at pH 6–7 (Galliard & Chan, 1980). The contrast in the optimum pH of winged bean lipoxygenase found in the present study and the values of the 5·8 and 6·0, found for the major isoenzymes of winged beans (Truong *et al.*, 1982*b*), suggests that considerable variability can occur in lipoxygenase from different varieties of winged bean.

Thermal inactivation of winged bean lipoxygenase

Whole winged bean seeds with a moisture content of 9·8% were boiled in water for 10 min. This reduced the lipoxygenase activity from 10 000 units

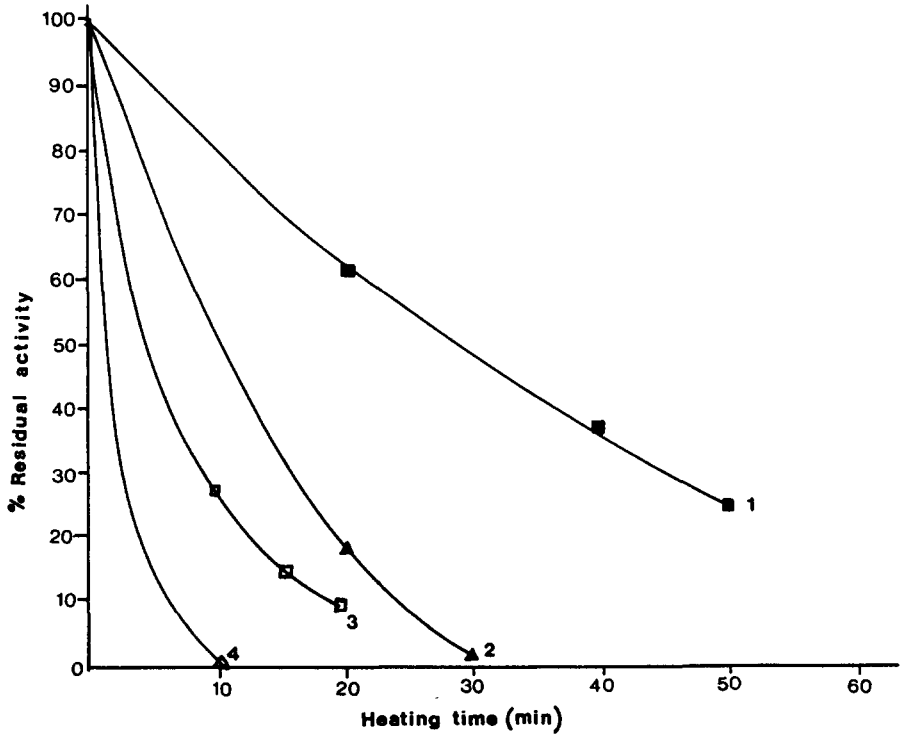

Fig. 2. Effect of heating winged bean seeds at 100°C on the lipoxygenase (L) and peroxidase (P) activities. (1–P, dry heat; 2–L, dry heat; 3–P, boiling; 4–L, boiling).

g^{-1} to zero, whereas 30 min heating in an oven at 100°C was required to reduce the activity to a value close to zero (Fig. 2).

The peroxidase in winged beans was considerably more stable than the lipoxygenase, with 27·3% of the peroxidase activity remaining after boiling in water for 10 min, and 45·1% peroxidase activity remaining after dry heating for 30 min at 100°C. Trypsin inhibitor activity also remained high after both these treatments, particularly dry heating, but nitrogen extractability was considerably reduced (Fig. 3).

The initial peroxidase activity of the winged bean seeds was 4347 units g^{-1}. The peroxidase activity of a potato tuber, which was included as a control, was 9782 units g^{-1} which is similar to the value of 10 875 units g^{-1} found by Mihalyi & Vamos-vigyazo (1975).

Heating the seeds for 20 min at 80°C or lower temperatures in an oven did not cause a large reduction in lipoxygenase activity (Table 1). The low moisture content (9·8%) of the seeds is important in the high stability of the enzyme towards dry heat. When the lipoxygenase was extracted from the winged beans and heated in aqueous solution at temperatures of 50°, 60° and

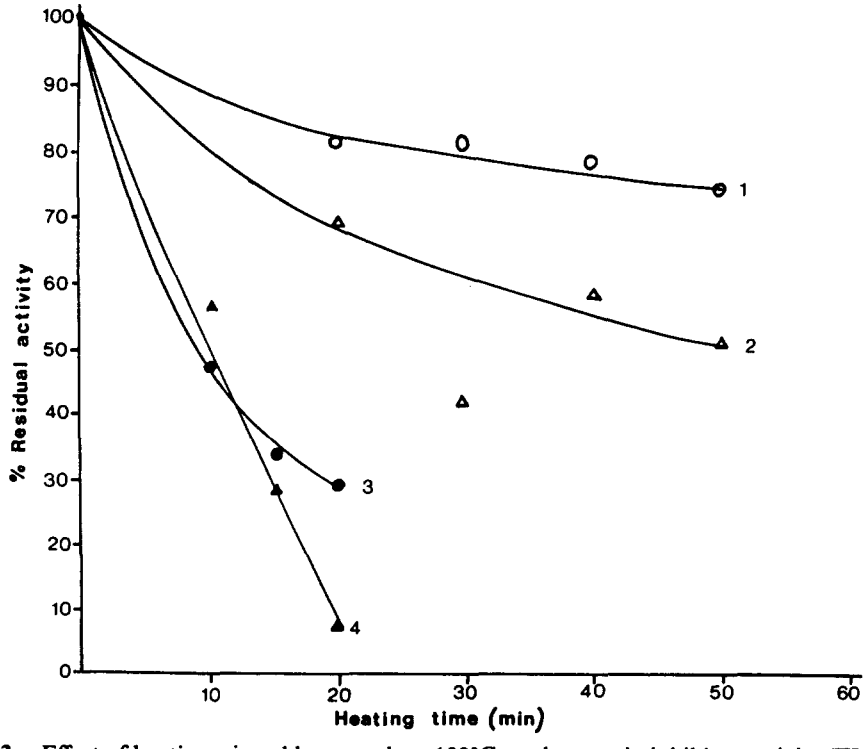

Fig. 3. Effect of heating winged bean seeds at 100°C on the trypsin inhibitor activity (TIA) and nitrogen extractability (NE). (1–TIA, dry heat; 2–NE, dry heat; 3–TIA, boiling; 4–NE, boiling).

TABLE 1
Effect of Dry Heat[a] on Lipoxygenase Activity of Winged Bean Seeds

Heating temperature, °C	—	60	80	100
Lipoxygenase activity (units g^{-1} defatted flour)	11 750	10 750	10 500	2 500

[a] Samples were heated for 20 min at the stated temperature.

65°C, inactivation was much more rapid (Fig. 4). Loss of 50% activity occurred in *ca* 5 min at 60°C, and only *ca* 1 min at 65°C. The winged bean lipoxygenase studied by Truong *et al.* (1982*b*) was considerably more stable than the extract in the present work with 50% activity being lost in *ca* 25 min at 65°C.

Crude winged bean lipoxygenase extracts studied by King & Puwastien (1984) were also more stable than the extract in the present work with 60 and 75% of the initial activity being retained by Suwan and Rajburi varieties after 180 min at 60°C.

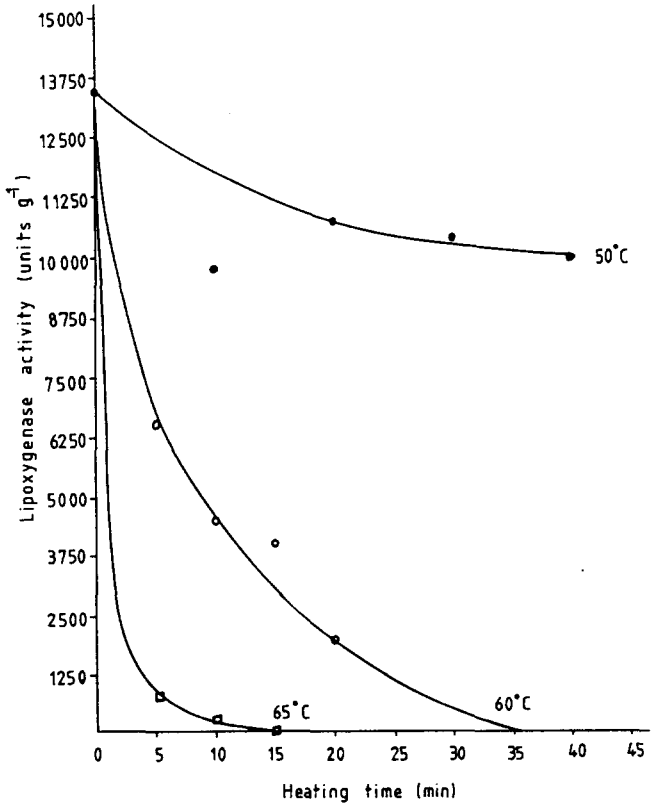

Fig. 4. Effects of heat on extracted winged bean lipoxygenase in aqueous solution.

Hence it appears that there are considerable variations in the properties of winged bean lipoxygenase studied by Truong *et al.* (1982*b*)., King & Puwastien (1984) and the present work. Differences in optimum pH and thermal stability have been observed.

Large differences in the thermal stability of lipoxygenase isoenzymes from other plants have been reported in the literature. Soybean lipoxygenase-1 lost half its activity in 25 min at 69°C, whilst soybean lipoxygenase-2 required only 0·7 min heating under these conditions (Christopher *et al.*, 1970). Hence it is possible that differences in the relative amounts of various isoenzymes lead to significant variability in the properties of lipoxygenase from winged bean varieties.

ACKNOWLEDGEMENTS

The authors wish to thank Mr Lee St Lawrence, Kins Plants Limited, for supplying the sample of winged beans, and the Commonwealth Scholarship Commission for a studentship for K. Mtebe.

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